

SUMMARY REPORT

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Kwang San Co., Ltd.

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List of abbreviations

BWMS	Ballast Water Management System
cm	Centimetre
m ³ /hr	Cubic Meters per Hour
°C	Degrees Celsius
cfu	Colony Forming Unit
DBP	Disinfection By-product
DNA	Deoxyribonucleic acid
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EC ₅₀	Effect Concentration, 50%
EPA	(U.S.) Environmental Protection Agency
g	Gram
g/h	Gram per Hour
HAAs	Haloacetic Acids
•OH	Hydroxyl Radical
HOBr	Hypobromous Acid
HOCl	Hypochlorous Acid
ISO	International Standard Organization
Koc	Partition Coefficient
L	Litre
LC ₅₀	Lethal Concentration, 50%
LOEC	Lowest Observed Effect Concentration
LP	Low-pressure
MP	Medium-pressure
µm	Micrometer
mg	Milligram
mJ/cm ²	Milli Jule per Centimeter Square
mV	Millivolt
mW s/cm ²	Milli Watt Second per Centimeter Square
nM	Nanomole
N.D	Not Detected
NOEC	No Observed Effect Concentration
NO ₃ ⁻	Nitrate
NO ₂ ⁻	Nitrite
NOM	Natural Organic Matter
NTU	Nephelometric Turbidity Unit
OECD	Organization for Economic Cooperation and Development
ORP	Oxidation Reduction Potential
POC	Particulate Organic Carbon
psi	Pounds per Square Inch
RNA	Ribonucleic acid

THMs	Trihalomethanes
TOC	Total Organic Carbon
TRO	Total Residual Oxidant
TSS	Total Suspended Solids
WHO	World Health Organization

1. INTRODUCTION

BioViolet™ is a BWMS developed by Kwang San Co., Ltd. BioViolet™ is composed of filtration system, UV system using a medium pressure ultraviolet (MPUV) lamp, and control system that includes power panel. Since this system mechanically and physically disinfects aquatic organisms, there is no secondary pollution of the sea by by-products and corrosion of the hull. There is no risk to the crew from by-products.

Ballast water is treated during ballasting and de-ballasting processes. During ballasting operation, most of aquatic organisms and particles larger than 50 µm in the ballast water are strained out through the filter. Also, aquatic organisms that were not removed by the filtration process are disinfected while passing by the UV system. In addition, to remove any aquatic organisms that may have survived in the ballast tank through the ballasting process, UV system is used once again to treat the ballast water during de-ballasting process.

The UV system does not include chemical elements. Therefore, BioViolet™ is a chemical-free disinfection system.

Table 1. Component and operation mode of BioViolet™

Item	Details
Product name	BioViolet™
Components	Filtration system + UV system + Control system
Operation mode	Ballasting mode, De-ballasting mode

2. PRINCIPLES OF ACCEPTABILITY OF THE BioViolet™

2.1 Introduction of the UV technology

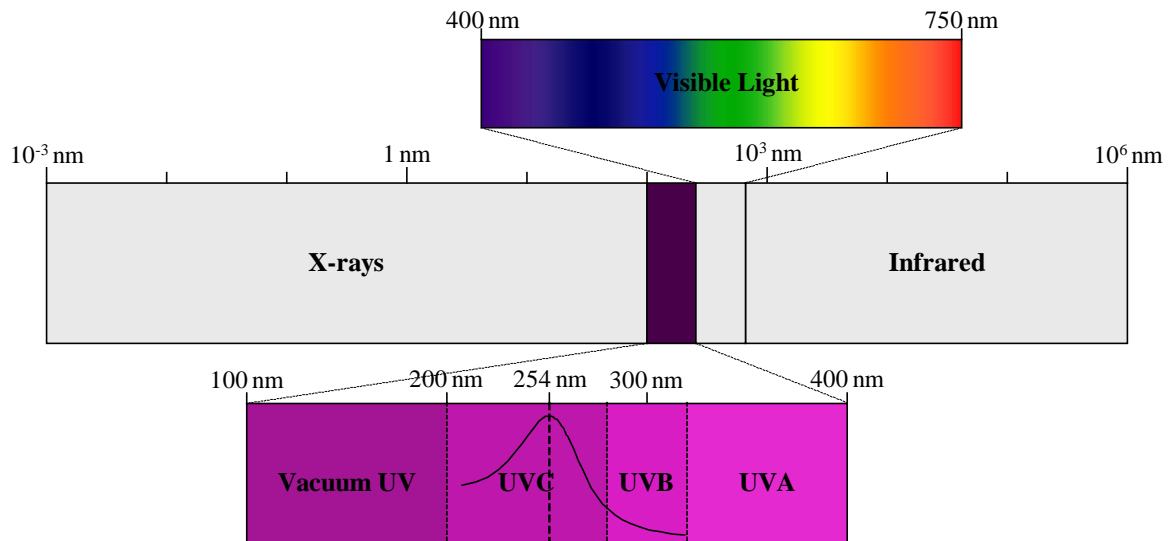


Figure 1. UV light in the Electromagnetic spectrum

UV light has wavelengths between 100~400 nm, longer than x-ray and shorter than visible light. UV light is classified into vacuum UV between 100~200 nm, UVC between 200~280 nm, UVB between 280~320 nm, and UVA between 320~400 nm according to wavelength (Figure 1) (Ultraviolet Germicidal Irradiation Handbook, 2009). Looking at the DNA (deoxyribonucleic acid) absorption wavelength and UV light range in Figure 1, UVA is not used for sterilization because DNA can hardly absorb UVA. Though it has enough energy for inactivation of microorganisms, short wavelength of vacuum UV causes low penetration in water. Therefore, it is inappropriate for use as main germicidal wavelength that directly influences disinfection of organisms. For such reasons, ultraviolet in UVC and UVB regions are used in many application fields of microorganism disinfection including water treatment. Such UV light directly and indirectly damages DNA. DNA of organisms absorb UV light of wavelength between 200~300 nm, especially 260 nm (Figure 2) (Zimmer and Slawson, 2002). UV radiation is absorbed by nucleic acids and proteins, which can cause photodamage and conformational changes, and subsequently disturb vital metabolic functions such as transcription, DNA replication, and translation (Holzinger and Lutz, 2006). In addition, UV light forms highly reactive oxidants to inflict indirect damage on DNA (Griffiths *et al.*, 1998).

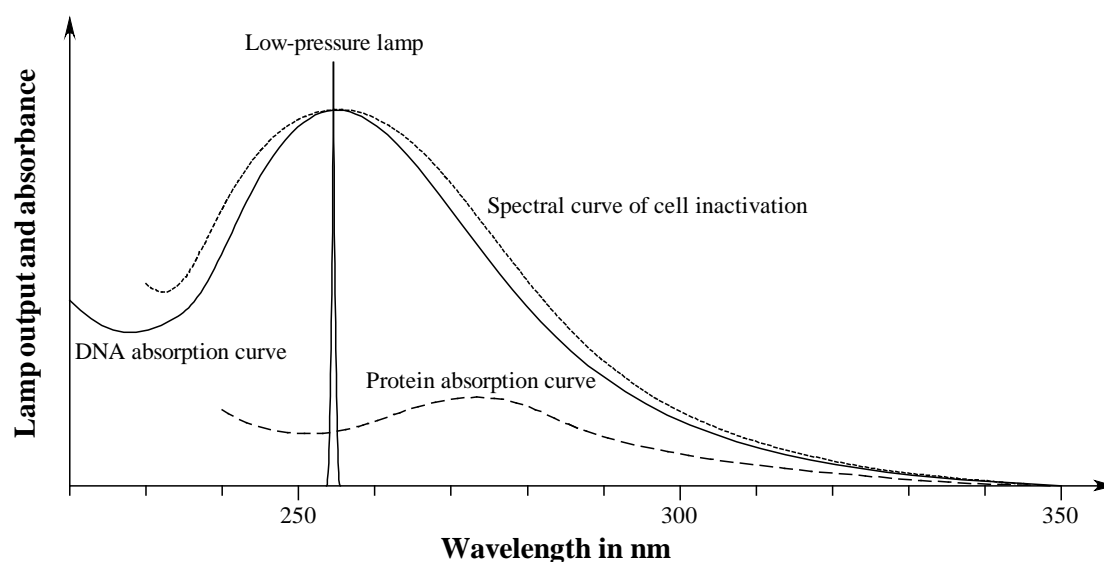


Figure 2. UV absorbance of DNA and protein

2.2 UV lamp

In UV technology for disinfection of microorganisms, care is required in selection of UV lamp since it has great effect on system specification such as footprint, power and disinfection efficacy. There are various types of UV lamp as listed below.

- Low-pressure (LP) mercury vapor lamps
- Low-pressure/high-output (LPHO) mercury vapor lamps
- Medium-pressure (MP) mercury vapor lamps
- Electrode-less mercury vapor lamps
- Metal halide lamps
- Xenon lamps (pulsed UV)
- Eximer lamps
- UV lasers
- Light emitting diodes (LEDs)

In general, LP, LPHO or MP mercury vapour lamp is mainly used in water treatment processes. Table 2 and 3 compare characteristics of such lamps.

Table 2. Lamp characteristics

Parameter	Low-pressure	Low-pressure/ high-output	Medium-pressure
Germicidal UV light	Monochromatic at 254 nm	Monochromatic at 254 nm	Polychromatic, including germicidal range
Mercury vapor pressure (Pa)	Approximately 0.93 (1.35×10^{-4} psi)	0.18~1.6 (2.6×10^{-5} ~ 2.3×10^{-4} psi)	40,000~4,000,000 (5.80~580 psi)

Operating temperature (°C)	Approximately 40	60~100	600~900
Electrical input (W/cm)	0.5	1.5~10	50~250
Germicidal UV output (W/cm)	0.2	0.5~3.5	5~30
Electrical to germicidal UV conversion efficiency (%)	35~38	30~35	10~20
Arc length (cm)	10~150	10~150	5~120
Relative number of lamps needed for a given dose	High	Intermediate	Low
Lifetime (hr)	8,000~10,000	8,000~12,000	4,000~8,000

Note: Information in this table was compiled from UV manufacturer data

Table 3. Comparison of UV technologies in ballast water treatment application

Parameters	Low-pressure & Low-pressure high-output		Medium-pressure	
	Rating	Reason	Rating	Reason
Footprint	Poor	High number of lamps	Good	Low number of lamps
Pressure loss	Poor	High number of lamps	Good	Low number of lamps
Efficiency (Germicidal)	Moderate	Monochromatic action spectrum, 254 nm	Good	Polychromatic action Spectrum, Each microorganism has a unique action spectrum. Damage to other molecules than DNA, e.g., enzymes
Reliability	Good	Low operating temperature. High lamp life	Moderate	High operating temperature. Average lamp life.
Power consumption	Low	High UVC output at 254 nm	Moderate	High UVC output in the range of 200~300 nm

Table 2 shows that UV intensity is higher in MP lamp compared to LP and LPHO lamps. Also, MP lamp emits polychromatic UV spectrum and therefore is most effective for BWMS in which large amount of various aquatic organisms must be disinfected.

Experimental results on disinfection of organisms with UV dose are given by the literature below.

A study of the ability of UV and free chlorine to disinfect a virus-containing ground water showed that UV is a more potent virucide than free chlorine, even after the chlorine residual was increased to 1.25 mg/L at a contact time of 18 minutes (Slade *et al.*, 1986). The UV dose used in this study was 25 mW·s/cm². Protozoan (oo)cysts, in particular *Giardia* and

Cryptosporidium, are considerably more resistant to UV inactivation than other microorganisms.

Even though protozoa were once considered resistant to UV radiation, recent studies have shown that ultraviolet light is capable of inactivating protozoan parasites. However, results indicate that these organisms require a much higher dose than that needed to inactivate other pathogens. Less than 80 percent of *Giardia lamblia* cysts were inactivated at UV dosages of 63 mW·s/cm² (Rice and Hoff, 1981). A 1-log inactivation of *Giardia muris* cysts was obtained when the UV dose was increased to 82 mW·s/cm² (Carlson *et al.*, 1982).

To achieve 2-log inactivation of *Giardia muris* cysts, a minimum ultraviolet light dose of above 121 mW·s/cm² is needed. Karanis *et al.*, (1992) examined the disinfection capabilities of ultraviolet light against *Giardia lamblia* cysts extracted from both animals and humans (Karanis *et al.*, 1992). Both groups suffered a 2-log reduction at UV doses of 180 mW·s/cm².

- Source: Alternative disinfectants and oxidants guidance manual (USEPA, 1999).

2.3 Mechanisms of microbial inactivation by UV light

Unlike chemical treatment using chlorine, disinfection of organisms by UV light is a physical disinfection technology. This is done by direct damage from DNA and RNA damages caused by UV irradiation and indirect damage from highly reactive oxidants formed by reaction of UV light with organic and inorganic matters in water.

Nucleic acid is in charge of metabolism and reproduction in organism. In general, nucleic acid is classified into DNA and RNA (ribonucleic acid). They are composed of nucleotide arrays with single or double structure. Nucleotide is composed of bases of purines and pyrimidines, sugar, and phosphate. Nucleotides of DNA and RNA differ in terms of base configuration. While purine bases are identical (adenine and guanine), pyrimidine bases differ (DNA: thymine and cytosine; RNA: uracil and cytosine). Commonly, DNA absorbs UV light with wavelength between 200~300 nm, especially 260 nm (Zimmer and Slawson, 2002).

In the mechanism of microbial inactivation by UV light, UV light irradiated on organisms is absorbed by the nucleotide in DNA. DNA exposed to UV light is damaged in various forms. Representative of such damages is damage from formation of photoproducts such as CPDs (cyclobutane pyrimidine dimers) and (6-4) PPs (pyrimidine-pyrimidone (6-4) photoproducts). CPDs is the most common form of DNA damage by UV light, where double bonding of base structure within DNA is destroyed and two adjacent pyrimidine bases on the same strand are bonded. (6-4) PPs are similar to pyrimidine dimers and form on the same sites. They disrupt the activity of DNA polymerase, thereby suppressing transcription and replication of DNA, and can result in inactivation of organisms (Macfadyen *et al.*, 2004; Li *et al.*, 2006). CPDs generally account for the majority of photoproducts formed (80~90 %), (6-4) PPs can be up to 300 times more effective in blocking DNA polymerase, and therefore more cytotoxic than CPDs (Mitchell and Nairn, 1989).

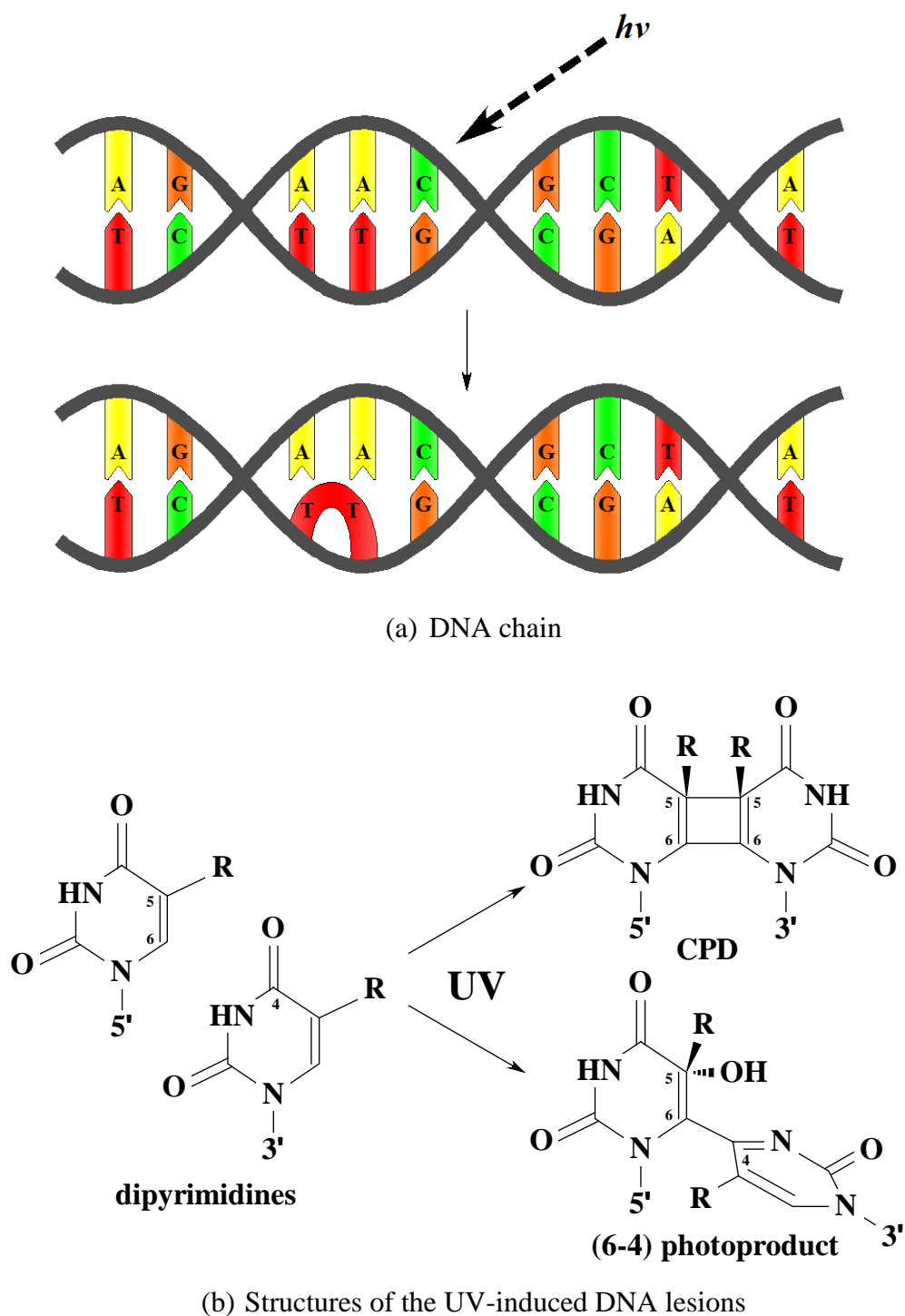
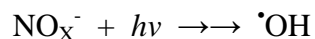
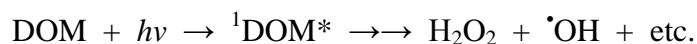


Figure 3. Formation of photoproducts by UV light
(The Ultraviolet Disinfection Handbook, 2008)

DOM (dissolved organic matter) in sea water is present at concentrations of about 0.5~2 mg/L (MacKinnon, 1981). DOM appears to be the main source for $\cdot\text{OH}$ (hydroxyl radical) over most of the oceans, but in upwelling areas nitrite and nitrate photolysis may also be important. DOM absorbs light, forms single photo-excited dissolved organic matter ($^1\text{DOM}^*$), and

makes $\cdot\text{OH}$ and H_2O_2 through reactions like photolysis, photoionization, and quenching. Reaction formulae are as follows.

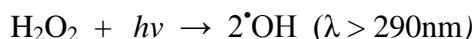


Hydrogen peroxide (H_2O_2) is the most prevalent reactive intermediate found in natural water systems, with observed concentrations of 14~290 nM in sea water and 100~7,000 nM in surface fresh water. This is due to its relative stability in most surface water, allowing for accumulation. *In situ* photochemical generation seems to be a significant source of H_2O_2 . Even though H_2O_2 is a powerful oxidant, H_2O_2 does not directly affect the oxidation of organic compounds because of its relative inertness in surface waters.

- Source: Natural water photochemistry-Singlet oxygen production and the degradation of dissolved organic nitrogen and organic pollutants (Edhlund, 2008)

H_2O_2 forms $\cdot\text{OH}$ through fenton reaction and absorption of light. The primary photolysis of H_2O_2 in aqueous solution yields $\cdot\text{OH}$. However, in nature water, H_2O_2 has very little absorption at wavelengths greater than 290 nm. The photochemical decay rate of H_2O_2 is slow in nature waters and $\cdot\text{OH}$ yield are small compared to other photochemical sources of $\cdot\text{OH}$ (e.g. DOM, nitrate, nitrite).

- Source: Reactive oxygen species in aquatic ecosystems (Kieber *et al.*, 2003)



$\cdot\text{OH}$ is a highly potent oxidizing free-radical species (Larsen and Berenbaum, 1988) that inactivates organisms by damaging DNA, proteins and cell membranes. $\cdot\text{OH}$ has a very short lifetime, typically in nanoseconds (Cooper *et al.*, 1988), and steady-state concentrations and production rates in sea water and freshwater are as described in Table 4 below.

Table 4. Measured and estimated $\cdot\text{OH}$ steady-state concentrations and production rates in sunlight-irradiated sea water and fresh water; n, number of samples used for the experiment; UD, undetectable; N.D., not determined (Mopper and Zhou, 1990).

Sample	$[\cdot\text{OH}]_{\text{ss}}^*$ $\times 10^{-18} \text{ M}$	$\cdot\text{OH}$ production rate [*] $\times 10^{-12} \text{ M/s}$ (nM/hour)	$\cdot\text{OH}$ production from different sources (%)			
			NO_3^-	NO_2^-	H_2O_2	Other (DOM)
			(Concentration of sources, μM) †			
Open-ocean surface water (Sargasso Sea, n = 6)	1.1 ± 0.1	2.8 ± 0.2 (10.1)	< 1 (< 0.05)	UD	< 4 (< 0.05)	> 95 (200)
Gulf Stream surface water (n = 1)	1.2	3.1 (11.2)		N.D.		
Deep-ocean water (Sargasso sea, > 700 m, n = 7)	6.3 ± 0.3	15.9 ± 0.7 (57.2)	19 (10)	1 (0.01)	3 (0.1)	77 (70)
Deep Gulf stream water (700 m, n = 1)	5.8	14.7 (52.9)		N.D.		
Subtropical coastal water (Biscayne Bay, FL, hightide, n = 4)	9.7 ± 1.2	24.4 ± 3.0 (87.8)	2 (2.0)	UD	2 (0.2)	96 (300)
Subtropical coastal water (Biscayne Bay, FL, lowtide n = 5)	13.7 ± 1.7	34.5 ± 4.3 (124.2)		N.D.		
Temperate coastal water (Vineyard Sound, MA, n = 1)	10.6	26.5 (95.4)		N.D.		
Equatorial upwelled water (estimated)	7.4	18.6 (67.0)	3 (5)	25 (0.2)	3 (0.1)	65 (200)
Coastal upwelled water (estimated)	26.3	66.1 (238)	7 (5)	35 (1)	6 (0.1)	52 (300)
10% Everglades water in Biscayne Bay water (n = 1)	30.1	68.9 (248)		N.D.		
DOM-rich freshwater (Everglades, n = 2)	$840 \ddagger$	420 ± 58 (1.5×10^3)		N.D.		

* $\pm 1\sigma$ SD.

† Concentrations of H_2O_2 and DOM (mole carbon basis) were estimated from published values (Zika *et al.*, 1985).

‡ This steady-state concentration was calculated with a measured scavenging coefficient of $5 \times 10^5 \text{ S}^{-1}$ (Zafiriou, 1983).

2.4 Microbial repair

Most microorganisms have repair mechanisms to repair damages by UV light. Repair mechanisms are classified into photoreactivation and dark repair (Knudson, 1985).

BWMS that uses UV technologies disinfects ballast water using UV light once again during de-ballasting operation in order to remove any aquatic organisms that may remain in the ballast tank due to such repairs.

2.4.1 Photoreactivation

In photoreactivation, enzymes energized by exposure to light between 310 and 490 nm (near and in the visible range) break the covalent bonds that form the pyrimidine dimers.

Knudson (1985) found that bacteria have the enzymes necessary for photoreactivation. Unlike bacteria, viruses lack the necessary enzymes for repair but can repair using the enzymes of a host cell (Rauth, 1965).

However, photoreactivation can be prevented by keeping the disinfected water in the dark for at least two hours before exposure to room light or sunlight (USEPA, 2006). Since ballast water is stored in the light-blocked ballast tank via main pipe after UV irradiation, photoreactivation does not occur.

2.4.2 Dark repair

Nucleotide excision repair is a more complex repair process that involves the coordination of numerous enzymes to remove damaged DNA. The process of repair through nucleotide excision is often referred to as dark repair because visible light is not required for the reaction (Yoon *et al.*, 2007).

BWMS using UV technologies disinfects the ballast water once again using the UV system during de-ballasting operation, considering possibility of dark repair while aquatic organisms in the treated water are stored in the ballast tank.

3. DESCRIPTION OF THE BioViolet™ AND ITS COMPONENTS

Refer to “Appendix 1” for detailed system description.

3.1 The process of the BioViolet™

Ballast water is treated during ballasting and de-ballasting operation. During ballasting operation, most of aquatic organisms and particles larger than 50 µm in the ballast water are strained out through the filter. Also, aquatic organisms that were not removed by the filtration process are disinfected while passing through the UV system. In addition to remove aquatic organisms that may have survived in the ballast tank after ballasting operation, the ballast water is treated once again using the UV system during de-ballasting operation. Figure 4 and Figure 5 below show schematic diagrams of BioViolet™ in ballasting and de-ballasting operation.

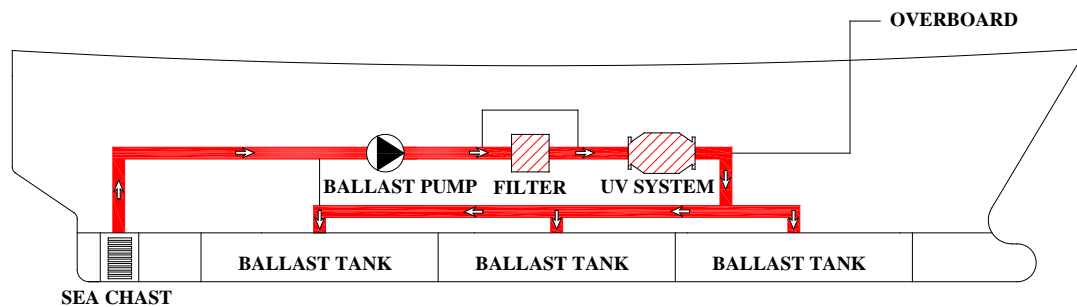


Figure 4. Schematic process diagram of the BioViolet™ in ballasting operation

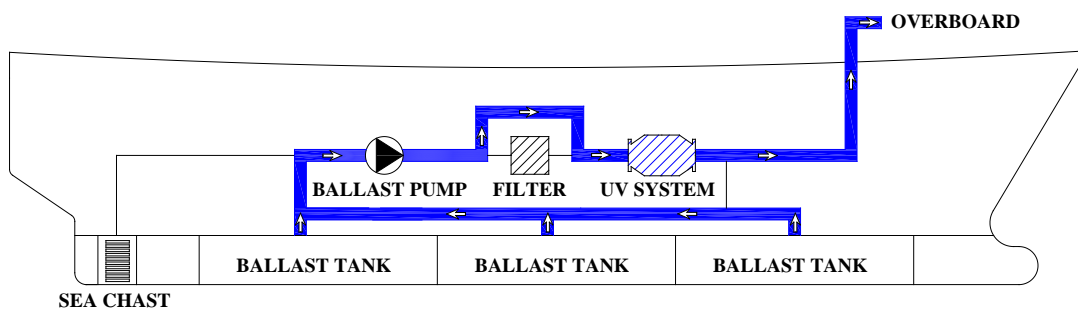


Figure 5. Schematic process diagram of the BioViolet™ in de-ballasting operation

3.2 The components of the BioViolet™

Since BioViolet™ does not accompany chemical treatments, it is an eco-friendly system harmless to the crew and environment. This is composed of filtration system, UV system, and control system that includes power panel. Appendix 1 provides detailed description on BioViolet™.

3.2.1 Filtration system

Filtration system is composed of components like filter housing, filter elements, sensors, motor, and back-flushing pump. Filter that used an element with slot size of 50 µm removes aquatic organisms and particles that came in through the ballast pump larger than 50 µm, while at the same time preventing breakage of quartz tubes that protect the UV lamp in the UV system. Sediment that influence UV penetration are removed during filtration process. Since physical impact is inflicted on aquatic organisms passing through the filter, overall disinfection efficiency of BioViolet™ is increased. Also, cleaning interval for the ballast tank is increased by reduced quantity of sediment in the ship's ballast tank with use of filter.

Filtration system is installed between ballast pump and UV system. It is only used during ballasting operation of BioViolet™ and by-passed during de-ballasting operation.

Though filtration system of BioViolet™ is designed with sufficient filtration area, aquatic organisms and particles that enter the filter through ballast pump larger than 50 µm are collected inside the filter elements, causing increase in differential pressure between inlet and outlet of the filter. Filtration system detects this differential pressure and automatically begins cleaning process once the value of differential pressure reaches the set point. Such process is referred to as automatic back-flushing. Set point is adjustable depending on installation conditions of the ship.

In automatic back-flushing process, back-flushing water is returned to the sea *in situ*.

3.2.2 UV system

UV system is composed of components such as chamber body, medium pressure UV lamps, quartz tubes, cleaning device, and various sensors. During ballasting process, the UV system is used to disinfect aquatic organisms that had not been removed in the filtration process. Also during de-ballasting process, the filter is by-passed during discharge of treated water, but the UV system is used once again to disinfect aquatic organisms that are not removed during ballasting process or may recover while treated water is being stored in the ballast tank.

Medium pressure/high intensity lamp was selected for the UV system to significantly reduce the quantity of lamp. Accordingly, maintenance was made easier. In addition, the chamber that can either be installed horizontally or vertically was designed with a special shape to greatly reduce its footprint. The UV system does not include chemical elements and therefore is a chemical-free disinfection system.

3.2.3 Control system

The control system controls and monitors the entire BioViolet™. The control system saves all records in the operation, exchanges important data including alarms with the central control system through communication, and controls power supplied to all components. In water treatment using UV light, parameters that influence disinfection include type of aquatic organisms, UV transmittance, treatment flow, lamp, water temperature, and etc. Here, UV transmittance is the parameter with largest influence on disinfection of aquatic organisms, given identical treatment flow and lamp output. Parameters influencing UV transmittance such as chromaticity and turbidity cannot be measured using a single sensor. The control system of BioViolet™ uses UV intensity acquired through two UV intensity sensors to mathematically compute UV dose and water quality inside the chamber, control power of the lamp for disinfection of aquatic organisms at optimal energy efficiency.

3.3 Land-based test facility

The land-based test facility for BioViolet™ developed by Kwang San Co., Ltd. is located in the fishery science technology center, Goseong-gun, Gyeongsangnam-do, Republic of Korea. The land-based test facility for BioViolet™ was set up to achieve type approval by the Ministry of Land, Transport and Maritime Affairs in accordance with the provisional regulation of type approval of ballast water management system by the Ministry of Land, Transport and Maritime Affairs (PR. No. 2011-37) / Guidelines for approval of ballast water management systems (G8, Res.MEPC.174 (58)) and in accordance with the procedure for approval of ballast water management systems that make use of active substances (G9).

Test facility is composed of 250 m³ capacity tanks, ballast pump, flow meter, pressure meters, level transmitters, level switches, sea water pump, sampling ports, sampling tanks, agitators, pipe, and main control panel. The main controller of the test facility, which corresponds to the ship's central control system, and the control system of BioViolet™ automatically perform all processes through mutual communication. Also, all data in system operation are logged. Figure 6 shows the land-based test facility.



Figure 6. Land-based test facility

3.3.1 Facility operation

The test facility repeats intake of sea water (or brackish water) for each test. The control system of the test facility uses flow information acquired from flow meter during ballasting and de-ballasting processes for real-time control of ballast pump to maintain flow of main pipe at 250 m³/h during each operation process.

For testing of brackish water and sea water, the UV system was operated twice for each test at maximum power to conduct chemical analysis, aquatic toxicity test and efficacy test under maximum UV dose. Also for remaining tests, power of UV lamp was controlled to maintain constant UV intensity value acquired from UV intensity sensors for conducting efficacy test.

Flow rate	250 ± 5 m ³ /h (no back-flushing)	
Treated volume	more than 200 m ³	
Treatments	Ballasting operation	Filter + UV system
	De-ballasting operation	UV system
Power consumption	max. 49 kW for UV system	
	max. 9.5 kW for filtration system	
	max. 1.5 kW for control system	
Salinity	Sea water	33.23~34.11 PSU
	Brackish water	19.77~20.31 PSU
UV transmittance	77~92 % (calculated value by two UV sensors)	
UV intensity control	UV sensor control	38~100 mW/cm ²
	max. power of UV system	49 kW

- Test preparation

Prior to ballasting operation, sea water that passed through strainer is filled in tank 1 and tank 2 by sea water pump. The amount of sea water filled differs according to the test condition (sea water or brackish water). In case of brackish water test, fresh water is additionally filled in each tank in order to adjust salinity of test water. Also to satisfy influent condition of IMO guidelines before the test, identical amounts of starch, glucose, silica granule, phytoplankton, zooplankton and heterotrophic bacteria are additionally added to tank 1 and tank 2.

In addition, agitator 1 of tank 1 and agitator 2 of tank 2 are operated during ballasting process, and agitator 2 of tank 2 and agitator 3 of tank 3 are operated during de-ballasting process, so that the test water in each tank is homogeneous.

- Ballasting operation

- Treated water

Ballasting operation on the treated water begins with “Ballasting (Treated) Mode ON” signal from the main controller of the test facility. Test water in tank 2 that satisfies the IMO guidelines passes through the filter and UV system by ballast pump and is transferred to tank 3. In this process, flow meter is used for real-time control of ballast pump to maintain flow of the main pipe at 250 m³/h.

In Figure 7, red line represents the transfer path of the treated water during ballasting operation. Sampling is performed three times by KOMERI during ballasting operation.

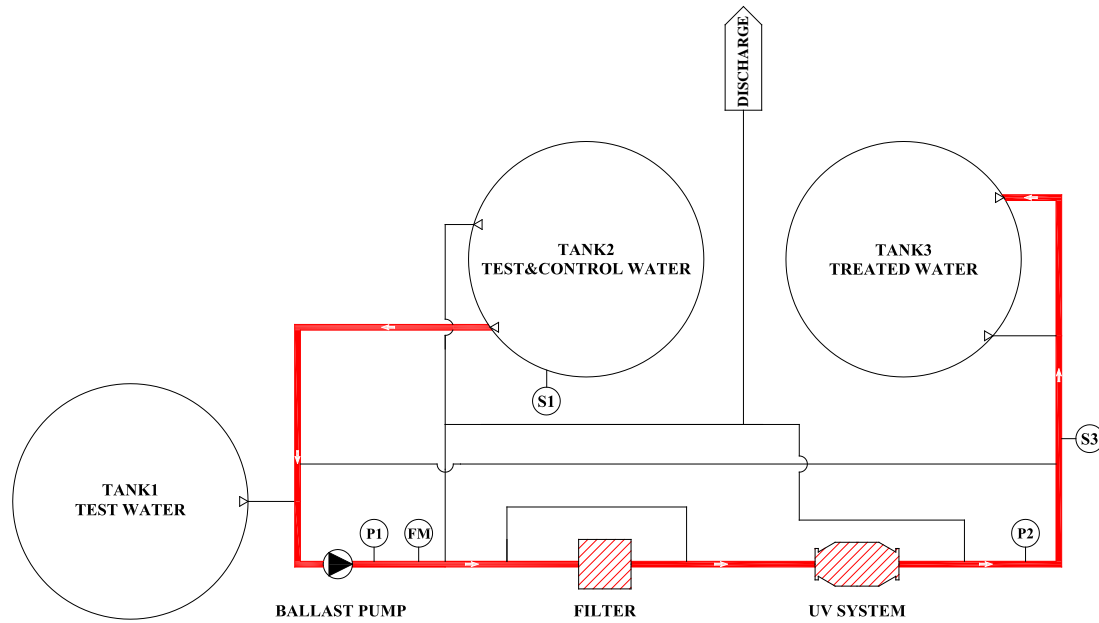


Figure 7. Ballasting operation of the treated water

- Control water

Ballasting operation on the control water begins by the main controller of the test facility after opening main pipe valves and controlling ballast pump. The test water is transferred from tank 1 to tank 2 by ballast pump. In this process, flow meter is used for real-time control of ballast pump to maintain flow of the main pipe at $250 \text{ m}^3/\text{h}$.

In Figure 8, blue line represents the transfer path of the control water during ballasting operation. Sampling is performed three times by KOMERI during ballasting operation.

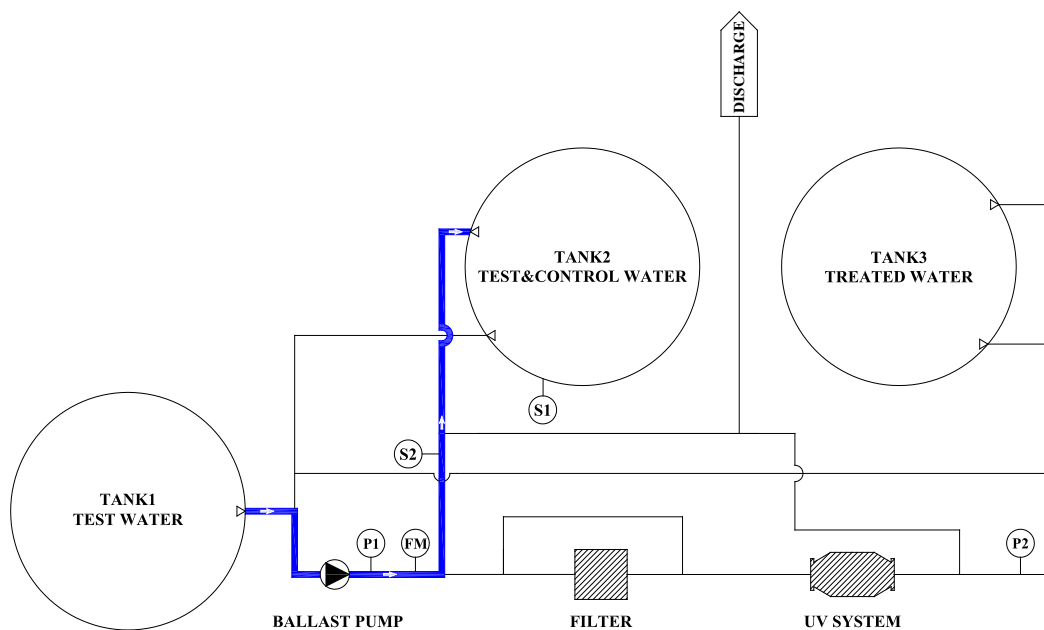


Figure 8. Ballasting operation of the control water

- Storage

The control water and treated water transferred by ballast pump that is operated by treatment rated capacity (TRC) are respectively stored in tank 2 and tank 3 for five days. During this storage period, light is completely blocked.

- De-ballasting operation

- Treated water

De-ballasting operation for the treated water begins with 'De-ballasting (Treated) Mode ON' signal from the main controller of the test facility. The treated water in tank 3 is discharged by ballast pump after passing by the UV system. In de-ballasting operation, filter is not used while the treated water is being discharged, but the UV system is used once again to disinfect any aquatic organisms that were not removed during ballasting process or can recover while the treated water is stored within ballast tank. In this process, flow meter is used for real-time control of ballast pump to maintain flow of the main pipe at 250 m³/h.

In Figure 9, red line represents the transfer path of the treated water during de-ballasting operation. Sampling is performed three times by KOMERI during de-ballasting operation.

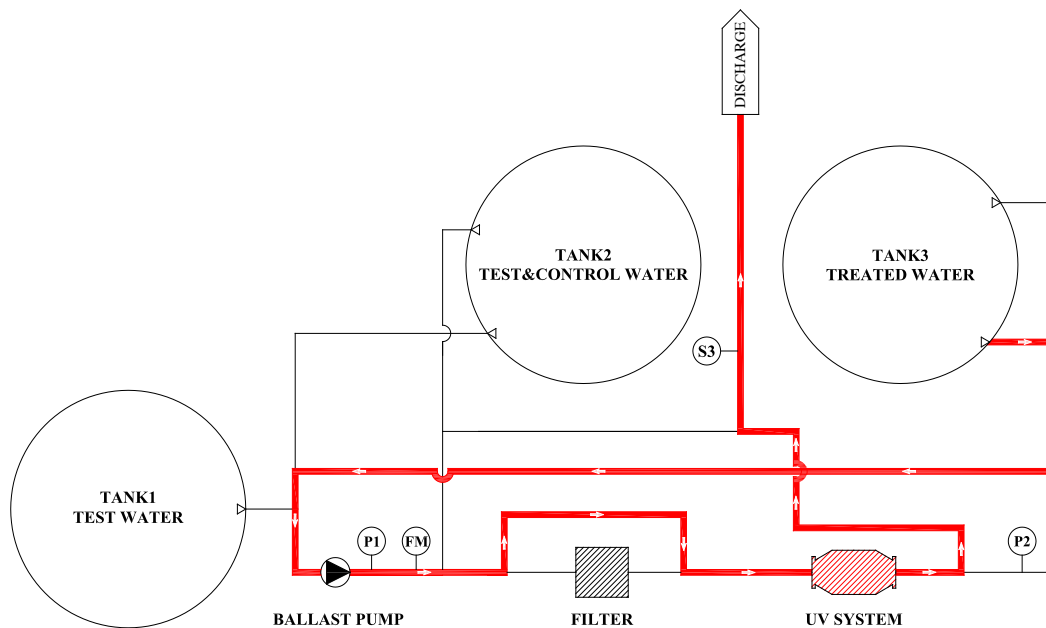


Figure 9. De-ballasting operation of the treated water

- Control water

De-ballasting operation of the control water begins by the main controller of the test facility after opening main pipe valves and controlling ballast pump. The control water in tank 2 is discharged by ballast pump. In this process, flow meter is used for real-time control of ballast pump to maintain flow of the main pipe at 250 m³/h.

In Figure 10, blue line represents the transfer path of the control water during de-ballasting operation. Sampling is performed three times by KOMERI during de-ballasting operation.



Sampling ports were installed for each water level of tank in order to check agitation status of test water and concentration of aquatic organisms. In addition to conduct three samplings for each operation process, sampling ports were installed on the main pipe. A sampling tank with automatic adjustment of sampling volume was installed for sampling of aquatic organisms larger than 50 μm from the treated water.

The schematic diagram illustrates the experimental setup. It features three main tanks: TANK1 (TEST WATER), TANK2 (TEST&CONTROL WATER), and TANK3 (TREATED WATER). TANK1 is connected to TANK2 via a line containing a pump (P1) and a flow meter (FM). TANK2 is connected to TANK3 via a line containing a valve (S1). TANK3 is connected to a DISCHARGE point via a line containing a valve (S3). A central vertical line connects the bottom of TANK2 to the bottom of TANK3, passing through a valve (S2). A horizontal line connects the bottom of TANK2 to the bottom of TANK3, passing through a valve (S2). A horizontal line connects the bottom of TANK3 to the bottom of TANK1, passing through a valve (P2). A horizontal line connects the bottom of TANK3 to the bottom of TANK2, passing through a valve (S3). A horizontal line connects the bottom of TANK3 to the bottom of TANK1, passing through a valve (S3).

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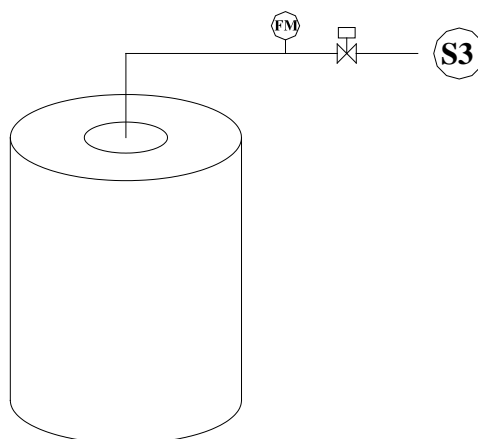


Figure 12. The diagram of the separately installed sampling tank.

All samples for chemical analysis, aquatic toxicity test, and efficacy test were collected from each sampling port. In Table 5, S1 represents test water sample, and S2 and S3 respectively represent control and treated water samples. Sampling on S2 and S3 was conducted three times, at the beginning, in the middle and at the end, during ballasting and de-ballasting operations. Each sample was sampled on Day 0 and Day 5 for efficacy test, on Days 0, 1 and 5 for chemical analysis, and on Day 5 for aquatic toxicity test.

Table 5. Sample information and sample tag for testing of the BioViolet™

Elapsed time(day)		0	1	5
Test water(S1)		KSU-S1D0-1*	-	-
Control water (S2)	B	KSU-S2BD0-1	KSU-S2D1-1	KSU-S2BD5-1
	M	KSU-S2MD0-1		KSU-S2MD5-1
	E	KSU-S2ED0-1		KSU-S2ED5-1
Treated water (S3)	B	KSU-S3BD0-1	KSU-S3D1-1	KSU-S3BD5-1
	M	KSU-S3MD0-1		KSU-S3MD5-1
	E	KSU-S3ED0-1		KSU-S3ED5-1
* cycle: -1, -2, -3, -4, -5, -6, -7, -8, -10, -11(high turbidity)				

Table 6. Sampling and organization

Test time	Organization		
	KOMERI (efficacy test)	KTR (chemical analysis)	MEI (aquatic toxicity test)
Day 0	S1, S2, S3	S1, S2, S3	-
Day 1	-	S2, S3	-
Day 5	S2, S3	S2, S3	S2, S3

Table 7. Sample information for efficacy testing of the BioViolet™

Sample for	Volume of sample ²	Remark
Test water ¹	1 L / 20 L / 1 L / 1 L	Basic water parameter: DO, ORP, pH, salinity, temperature, turbidity Viable organisms: $\geq 50 \mu\text{m}$, $\geq 10\sim 50 \mu\text{m}$ Bacteria: Heterotrophic bacteria, <i>E. coli</i> , intestinal Enterococci, toxicogenic <i>V. cholerae</i>
Treated water during ballasting on day 0	10 L / 1 m ³ / 2 L / 1 L	
Control water after ballasting on day 0	1 L / 20 L / 1 L / 1 L	
Treated water during de-ballasting on day 5	10 L / 1 m ³ / 2 L / 1 L	
Control water during de-ballasting on day 5	1 L / 20 L / 1 L / 1 L	

¹ Test water including organism ($\geq 50 \mu\text{m}$, $\geq 10\sim 50 \mu\text{m}$), starch, and glucose to correspond with G8.

² Marine organisms($\geq 10\sim 50 \mu\text{m}$) / Marine organisms($\geq 50 \mu\text{m}$) / Bacteria / DOC & POC

3.3.3 Test methods of the land-based test

All tests and analyses were performed in accordance with the G8 and the G9 by internationally accredited laboratories (ISO/IEC 17025). Table 8 shows analytical methods.

Table 8. Test methods

Item		Method
Chemical analysis		
TRO/FRO		ISO 7393-2: 1985
Bromate		ISO 15061: 2001
VOCs/THMs		US EPA 524.2: 1995
Halogenated phenols		US EPA 8041A: 2007
HANs		US EPA 551.1: 1995
HAAs		US EPA 552.2: 1995
TOC (DOC/POC)		ISO 8245: 1999
Aquatic toxicity test		
Growth inhibition test	Algae	ISO 10253: 2006
Acute/Chronic test	Invertebrate	ASTM E-1440-91: 2004 Janssen <i>et al.</i> , 1994
	Fish	OECD guidelines 203: 1992 OECD guidelines 212: 1992
Efficacy test		

Viable organisms	$\geq 50 \mu\text{m}$	Fleming & Coughlan, 1978 EPA 600/R-10/146: 2010 APHA standard method 10200 C: 2012
	$\geq 10\text{-}50 \mu\text{m}$	Anja <i>et al.</i> , 2005 APHA standard method 10200 C: 2012
Heterotrophic bacteria		APHA standard method 9215: 2012
<i>Escherichia coli</i>		US EPA 1603: 2009
Intestinal Enterococci		US EPA 1600: 2009
Toxicogenic <i>Vibrio cholerae</i> (O1, O139)		APHA standard method 9260 H: 2012

4. APPLICATION DATA SET

4.1 Efficacy test result of the BioViolet™

4.1.1 Test water condition and efficacy test results of the BioViolet™

Table 9. Land-based test schedule

Number of trial	Test date and operation mode	
	Ballasting operation	De-ballasting operation
3~32 PSU		
1 st	1 September 2011	6 September 2011
2 nd	28 September 2011	3 October 2011
3 rd	5 October 2011	10 October 2011
4 th	12 October 2011	17 October 2011
5 th	19 October 2011	24 October 2011
> 32 PSU		
6 th	26 October 2011	31 October 2011
7 th	2 November 2011	7 November 2011
8 th	9 November 2011	14 November 2011
9 th	16 November 2011	21 November 2011
10 th	23 November 2011	28 November 2011
Additional test (high turbidity)		
11 th	7 December 2011	12 December 2011

Table 10 shows the test water conditions. All efficacy tests were performed in accordance with the G8.

Table 10. Basic parameters of the test water

Number of trial	Salinity (PSU)	Temp. (°C)	pH	DO (mg/l)	ORP (mV)	Turbidity (NTU)
3~32 PSU						
1 st	20.31	25.18	8.51	7.48	279	25.00
2 nd	20.21	23.95	8.31	7.69	365	20.10
3 rd	19.77	21.61	8.28	7.84	343	20.60
4 th	20.14	21.56	8.54	7.90	272	23.80
5 th	19.84	20.07	8.53	8.06	304	23.00
> 32 PSU						
6 th	33.38	17.69	8.34	7.80	288	5.46
7 th	34.11	18.37	8.28	7.68	377	5.55
8 th	33.23	18.38	8.24	7.74	367	3.48
9 th	34.09	16.18	8.36	7.87	332	4.72
10 th	33.97	14.82	8.33	8.06	266	4.17
Additional test (high turbidity)						
11 th	34.01	13.32	8.18	8.30	360	122.00

The concentration of viable organisms, heterotrophic bacteria, DOC, POC, TSS and organism diversity in test water were satisfied the requirements of G8 (Table 11 and 12).

Table 11. Abundance of viable organisms, heterotrophic bacteria, and concentration of DOC, POC and TSS in test water

Number of trial	Viable organisms		Heterotrophic bacteria (cells/ml)	DOC (mg/l)	POC (mg/l)	TSS (mg/l)
	≥ 50 μm (inds./m ³)	≥ 10~50 μm (inds./ml)				
3~32 PSU						
1 st	249,334	1,930	34,864	6.99	10.18	86.40
2 nd	188,000	1,239	52,364	6.56	5.32	69.10
3 rd	147,000	2,830	23,455	6.22	6.17	73.60
4 th	127,834	2,730	12,318	6.52	7.44	75.90

5 th	121,167	2,242	59,000	6.54	7.69	72.70
> 32 PSU						
6 th	136,000	2,425	44,273	2.68	2.02	17.20
7 th	197,667	2,853	50,500	2.59	1.80	23.60
8 th	192,167	1,620	14,273	2.44	1.73	16.10
9 th	160,667	2,211	10,273	2.53	1.83	18.50
10 th	137,334	2,931	25,273	2.60	1.97	18.80
Additional test (high turbidity)						
11 th	131,000	2,623	11,773	1.55	1.75	267.70

Table 12. Viable organisms diversity in the test water

Number of trial	Viable organisms	
	≥ 50 μm	≥ 10~50 μm
3~32 PSU		
1 st	4 Phyla/Division 10 Species	3 Phyla/Division 5 Species
2 nd	4 Phyla/Division 9 Species	3 Phyla/Division 6 Species
3 rd	3 Phyla/Division 10 Species	3 Phyla/Division 9 Species
4 th	4 Phyla/Division 8 Species	3 Phyla/Division 5 Species
5 th	5 Phyla/Division 10 Species	4 Phyla/Division 10 Species
> 32 PSU		
6 th	4 Phyla/Division 10 Species	4 Phyla/Division 8 Species
7 th	4 Phyla/Division 11 Species	3 Phyla/Division 10 Species
8 th	5 Phyla/Division 13 Species	4 Phyla/Division 9 Species
9 th	4 Phyla/Division 10 Species	3 Phyla/Division 6 Species
10 th	3 Phyla/Division 7 Species	3 Phyla/Division 7 Species
Additional test (high turbidity)		
11 th	3 Phyla/Division 5 Species	3 Phyla/Division 5 Species

All efficacy test results of the discharge treated water were satisfied the regulation D-2 and the results of the control water were satisfied valid condition in the G8 requirements (Table 13 and 14).

Table 13. Viable organism of the control and discharge treated water

Number of trial	Viable organisms			
	$\geq 50 \mu\text{m}$ (inds./m ³)		$\geq 10\sim 50 \mu\text{m}$ (inds./ml)	
	Control water	Treated water	Control water	Treated water
3~32 PSU				
1 st	43,028	1	180	1
2 nd	38,278	0	362	1
3 rd	68,223	0	528	1
4 th	52,001	0	289	2
5 th	40,556	0	1,140	3
> 32 PSU				
6 th	41,751	0	414	1
7 th	86,945	0	452	1
8 th	27,612	0	353	1
9 th	15,945	0	653	1
10 th	30,889	0	1,501	7
Additional test (high turbidity test)				
11 th	22,278	0	633	3

Table 14. Viable organism of the control and the discharge treated water

Number of trial	Bacteria (CFU/100 ml)		
	<i>Escherichia coli</i>	Intestinal Enterococci	Toxicogenic <i>Vibrio cholerae</i> (O1, O139)
3~32 PSU			
1 st	0	1	0
2 nd	0	0	0
3 rd	0	0	0
4 th	0	0	0
5 th	0	0	0

> 32 PSU			
6 th	0	0	0
7 th	0	0	0
8 th	0	0	0
9 th	0	0	0
10 th	1	0	0
Additional test (high turbidity test)			
11 th	0	0	0

4.2 Identification of substances

4. 2. 1 Active substance (photon)

The UV system of BioViolet™ is a system that disinfects diverse aquatic organisms in sea water using polychromatic MPUV lamp for damaging their DNA and RNA.

Also, light is electromagnetic wave and has properties of wave and particle. Photon is a particle with a definite energy when reacting with substances.

Since the UV system of BioViolet™ disinfects organisms using “photon” radiated from the UV lamp, “active substance” in BioViolet™ is photon.

However, photon does not remain in the treated water that passes by the UV system, because photon reacts in the UV system for an extremely short period of time to damage DNA and RNA of aquatic organism.

4. 2. 2 Relevant chemicals (hydroxyl radical, $\cdot\text{OH}$)

DOM, nitrate (NO_3^-), and nitrite (NO_2^-) form hydroxyl radical ($\cdot\text{OH}$) by photochemical reaction with the UV light irradiated from the UV lamp. $\cdot\text{OH}$ is a strong oxidant with very short lifetime in nanoseconds. In addition, $\cdot\text{OH}$ has a potential to form new products in reaction with surrounding compounds immediately after formation. Such reaction products are parameters that influence aquatic environment. However, it is difficult to characterize the type of reaction products formed by reaction with $\cdot\text{OH}$ because of their diversity.

Though $\cdot\text{OH}$ is not directly discharged to the ocean ecosystem because of extremely short lifetime, reaction products that can be formed in reaction with $\cdot\text{OH}$ may be discharged to the ocean ecosystem. Hence, $\cdot\text{OH}$ is defined as a “relevant chemical.”

4. 2. 3 Other components

In sea water, various chemical compounds such as DOM, nitrate (NO_3^-), nitrite (NO_2^-), and chlorinated organic compounds exist. Such chemical compounds have a potential to form diverse reaction products like trihalomethanes (THMs), haloacetic acids (HAAs), and total organic halides by photochemical reaction and reaction with hydroxyl radical ($\cdot\text{OH}$).

The UV system of BioViolet™ defined all reaction products formed by photochemical reaction that can potentially be discharged to the sea as “other components.”

There are many literature reviews on “other components” that can be formed by UV irradiation.

Several studies have shown low-level formation of non-regulated DBPs(e.g., aldehydes) as a result of applying UV light at doses greater than 400 mJ/cm² to wastewater and raw drinking water sources (Liu *et al.*, 2002; Venkatesan *et al.*, 2003). At the doses typical for UV disinfection in drinking water (< 140 mJ/cm²), however, no significant change was observed (Kashinkunti *et al.*, 2003). UV disinfection has not been found to significantly increase the assimilable organic carbon (AOC) of drinking water at UV doses ranging from 18 - 250 mJ/cm² (Kruithof and van der Leer 1990; Akhlaq *et al.*, 1990; Malley *et al.*, 1996).

Trihalomethanes (THMs) and haloacetic acids (HAAs) are two categories of halogenated DBPs that EPA currently regulates. UV light at doses less than 400 mJ/cm² has not been found to significantly affect the formation of THMs or HAAs upon subsequent chlorination (Malley *et al.*, 1996; Kashinkunti *et al.*, 2003; Zheng *et al.*, 1999; Liu *et al.*, 2002; Venkatesan *et al.*, 2003).

- Source: Ultraviolet disinfection guidance manual for the final long term 2 enhanced surface water treatment rule (USEPA, 2006).

Table 15. Concentration of potential chemicals and density in brackish water test

Sampling time			0 day (2011. 10. 05)			1 day (2011. 10. 06)		5 day (2011. 10. 10)	
Sampling point / tank			Test water	Control	Treated	Control	Treated	Control	Treated
Sample tag			KSU-S1D0-3	KSU-S2D0-3	KSU-S3D0-3	KSU-S2D1-3	KSU-S3D1-3	KSU-S2D5-3	KSU-S3D5-3
Compounds	DL	Unit							
TRO	0.03	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
FRO	0.03	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ozone(O ₃)	0.13	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ClO ₂	0.02	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ClO	0.09	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sulfide(S ²⁻)	0.02	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromate ion(BrO ₃ ⁻)	0.08	μg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromide(Br ⁻)	0.01	mg/L	54.8	55.4	55.6	55.0	55.4	55.3	55.4
AOX	0.01	mg/L	0.02	0.03	0.04	0.03	0.02	0.02	0.02
DOC	0.08	mg/L	6.22	6.36	6.19	5.33	2.98	2.82	2.36
POC	0.08	mg/L	6.17	7.64	6.75	1.34	0.88	3.41	4.81
Organic chlorinated compound									
1,1-Dichloroethene	0.02	μg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dichloromethane	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
trans-1,2-Dichloroethene	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,1-Dichloroethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
cis-1,2-Dichloroethene	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromochloromethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Trichloromethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

1,2-Dichloroethane	0.01	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,1,1-Trichloroethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tetrachloromethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dibromomethane	0.05		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2-Dichloropropane	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dichlorobromomethane	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,1,2-Trichloroethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dibromochloromethane	0.01		1.92	1.88	N.D.	1.94	N.D.	1.81	N.D.
Tetrachloroethene	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,1,1,2-Tetrachloroethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Chlorobenzene	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tribromomethane	0.03		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,1,2,2-Tetrachloroethane	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,3-Trichloropropane	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromobenzene	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2-Chlorotoluene	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4-Chlorotoluene	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2-Dibromo-3-chloropropane	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,4-Trichlorobenzene	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,3-Trichlorobenzene	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,3,5-Tribromobenzene	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,4-Tribromobenzene	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
THMs			2.43	2.35	N.D.	1.94	N.D.	1.81	N.D.
Totals VOCs			2.43	2.35	N.D.	1.94	N.D.	1.81	N.D.
HANs									
Trichloroacetonitrile	0.01	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dichloroacetonitrile	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Chloral hydrate	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Chloropicrin	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromochloroacetonitrile	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dibromoacetonitrile	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sum HANs			N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
HAAs									
Monochloroacetic acid	0.24	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Monobromoacetic acid	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dichloroacetic acid	0.02		0.61	0.62	0.32	0.50	0.36	0.49	0.93
Dalapon	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Trichloroacetic acid	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromochloroacetic acid	0.02		N.D.	N.D.	0.46	N.D.	0.10	N.D.	0.12
Dibromoacetic acid	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromodichloroacetic acid	0.03		0.34	0.36	0.76	0.79	1.04	0.94	2.08
Tribromoacetic acid	0.24		6.84	6.48	6.57	7.68	6.61	8.09	6.58
Sum HAAs			7.78	7.46	8.10	8.98	8.12	9.52	9.72
Chlorinated phenols									
2-Chlorophenol	0.04	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3-Chlorophenol	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

4-Chlorophenol	0.04	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,6-Dichlorophenol	0.07		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,5-Dichlorophenol	0.09		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4-Dichlorophenol	0.05		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3,5-Dichlorophenol	0.03		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3-Dichlorophenol	0.07		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3,4-Dichlorophenol	0.03		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4,6-Trichlorophenol	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,6-Trichlorophenol	0.03		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4,5-Trichlorophenol	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,5-Trichlorophenol	0.03		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,4-Trichlorophenol	0.03		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3,4,5-Trichlorophenol	0.15		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,5,6-Tetrachlorophenol	0.05		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,4,6-Tetrachlorophenol	0.10		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,4,5-Tetrachlorophenol	0.12		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Pentachlorophenol	0.06		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromonated phenols									
2,6-Dibromophenol	0.04	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4-Dibromophenol	0.10		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4,6-Tribromphenol	0.13		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Density(20 ℃)	0.0001	g/cm³	1.0130	1.0130	1.0130	1.0130	1.0130	1.0130	1.0130

Table 16. Concentration of potential chemicals and density in sea water test

Sampling time			0 day (2011. 10. 26)			1 day (2011. 10. 27)		5 day (2011. 10. 31)	
Sampling point/tank			Test water	Control	Treated	Control	Treated	Control	Treated
Sample tag			KSU-S1D0-6	KSU-S2D0-6	KSU-S3D0-6	KSU-S2D1-6	KSU-S3D1-6	KSU-S2D5-6	KSU-S3D5-6
Compounds	DL	Unit							
TRO	0.03	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
FRO	0.03	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ozone(O ₃)	0.13	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ClO ₂	0.02	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
ClO	0.09	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sulfide(S ⁻²)	0.02	mg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromate ion(BrO ₃ ⁻)	0.08	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromide(Br ⁻)	0.01	mg/L	65.9	66.1	65.8	65.9	65.7	65.9	66.4
AOX	0.01	mg/L	0.06	0.07	0.09	0.04	0.05	0.04	0.04
DOC	0.08	mg/L	2.68	2.64	2.79	1.79	1.74	1.66	1.96
POC	0.08	mg/L	2.02	1.94	1.97	1.45	1.85	0.19	0.11
Organic chlorinated compound									
1,1-Dichloroethene	0.02	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dichloromethane	0.02		7.15	6.98	N.D.	0.17	N.D.	N.D.	N.D.
trans-1,2-Dichloroethene	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

1,1-Dichloroethane	0.01	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
cis-1,2-Dichloroethene	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromochloromethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Trichloromethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2-Dichloroethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,1,1-Trichloroethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tetrachloromethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dibromomethane	0.05		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2-Dichloropropane	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dichlorobromomethane	0.02		0.51	0.45	0.17	0.13	0.24	0.02	0.11
1,1,2-Trichloroethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dibromochloromethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tetrachloroethene	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,1,1,2-Tetrachloroethane	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Chlorobenzene	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tribromomethane	0.03		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,1,2,2-Tetrachloroethane	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,3-Trichloropropane	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromobenzene	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2-Chlorotoluene	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4-Chlorotoluene	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2-Dibromo-3-chloropropane	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,4-Trichlorobenzene	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,3-Trichlorobenzene	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,3,5-Tribromobenzene	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1,2,4-Tribromobenzene	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
THMs			0.51	0.45	0.17	0.13	0.24	0.02	0.11
Totals VOCs			7.66	7.43	0.17	0.30	0.24	0.02	0.11
HANs									
Trichloroacetonitrile	0.01	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dichloroacetonitrile	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Chloral hydrate	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Chloropicrin	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromochloroacetonitrile	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dibromoacetonitrile	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sum HANs			N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
HAAs									
Monochloroacetic acid	0.24	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Monobromoacetic acid	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dichloroacetic acid	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dalapon	0.04		0.32	0.34	0.41	0.28	0.40	0.27	0.32
Trichloroacetic acid	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromochloroacetic acid	0.02		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dibromoacetic acid	0.01		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Bromodichloroacetic acid	0.03		1.54	1.48	1.42	1.33	1.37	1.31	1.10
Tribromoacetic acid	0.24		0.63	0.53	1.27	1.24	1.13	1.52	1.13

Sum HAAs			2.49	2.35	3.09	2.85	2.90	3.09	2.54
Chlorinated phenols									
2-Chlorophenol	0.04	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3-Chlorophenol	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4-Chlorophenol	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,6-Dichlorophenol	0.07		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,5-Dichlorophenol	0.09		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4-Dichlorophenol	0.05		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3,5-Dichlorophenol	0.03		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3-Dichlorophenol	0.07		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3,4-Dichlorophenol	0.03		N.D.	N.D.	2.89	N.D.	N.D.	N.D.	N.D.
2,4,6-Trichlorophenol	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,6-Trichlorophenol	0.03		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4,5-Trichlorophenol	0.04		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,5-Trichlorophenol	0.03		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,4-Trichlorophenol	0.03		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3,4,5-Trichlorophenol	0.15		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,5,6-Tetrachlorophenol	0.05		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,3,4,6-Tetrachlorophenol	0.10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
2,3,4,5-Tetrachlorophenol	0.12	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Pentachlorophenol	0.06	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	
Bromonated phenols									
2,6-Dibromophenol	0.04	µg/L	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4-Dibromophenol	0.10		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2,4,6-Tribromphenol	0.13		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Density(20 ℃)	0.0001	g/cm³	1.0233	1.0232	1.0232	1.0233	1.0232	1.0233	1.0233

4.3 Data on effects on aquatic plant, invertebrate and fish including sensitive and representative organisms (G9: 4.2.1.1)

The most sensitive organism for the discharge treated water from the BioViolet™ was the fish , *Paralichthys olivaceus* exposed during 7 days (chronic toxicity test) that gave the LOEC value of 100.00 %, NOEC value of 50.00 %, 7d-LC₂₅ value of 80.50 % and 7d-LC₅₀ value of higher than 100.00 % for the > 32 PSU discharge treated water (Table 20). The results are summarized in Tables 17~21.

4.3.1 Growth inhibition test (Table 17)

Algae: Study to assess the effects of the discharge treated water by the BioViolet™ on the diatom, *S. costatum*, for 72 and 96 hours, NOECs, LOECs, 72h-EC₅₀ and 96h-EC₅₀ value based on the cell densities at the end of the experiment were calculated for > 32 and 3~32 PSU discharge treated water from the BioViolet™. > 32 and 3~32 PSU discharge treated water did not have toxic effect on the *S. costatum*

Table 17. Growth inhibition test results on > 32 and 3~32 PSU discharge treated water from BioViolet™

	Species	Test water (PSU)	NOEC	LOEC	LC ₅₀ and/or EC ₅₀		Reference
			Conc. (%)		End point	Conc. (%)	
Algae	<i>Skeletonema costatum</i>	> 32	100.00	> 100.00	72h-EC ₅₀	> 100.00	ISO 10253
			100.00	> 100.00	96h-EC ₅₀	> 100.00	
		3~32	100.00	> 100.00	72h-EC ₅₀	> 100.00	
			100.00	> 100.00	96h-EC ₅₀	> 100.00	

4.3.2 Acute aquatic toxicity (Table 18, 19)

Acute toxicity tests with the BioViolet™ > 32 PSU and 3~32 PSU water covering multiple test species (a fish, an invertebrate) indicated no acute toxicity to any test organisms at any concentrations of the discharge treated water as tested (see report in appendix 6). The results are summarized in Tables 18 and 19.

Invertebrate: Study to assess the effects of the discharge treated water from the BioViolet™ on the Rotifer, *Brachionus plicatilis*, for 24 hours, NOEC, LOEC and 24h-LC₅₀ values based on survival data at the end of the experiment were calculated for > 32 and 3~32 PSU discharge treated water from the BioViolet™. > 32 and 3~32 PSU discharge treated water did not have acute toxic effect on the Rotifer, *B. plicatilis*.

Fish: Study to assess the effects of the discharge treated water from the BioViolet™ on the olive flounder, *Paralichthys olivaceus*, for 96 hours, NOEC, LOEC and 96-LC₅₀ values based on survival data at the end of the experiment were calculated for > 32 and 3~32 PSU discharge treated water from the BioViolet™. > 32 and 3~32 PSU discharge treated water did not have acute toxic effect on the olive flounder, *P. olivaceus*.

Table 18. Acute aquatic toxicity of > 32 PSU of the discharge treated water from BioViolet™

	Species	NOEC	LOEC	LC ₅₀ and/or EC ₅₀		Reference
		Conc. (%)		End point	Conc. (%)	
Invertebrate	<i>Brachionus plicatilis</i>	100.00	> 100.00	24h-LC ₅₀	> 100.00	ASTM E1440-91
Fish	<i>Paralichthys olivaceus</i>	100.00	> 100.00	96h-LC ₅₀	> 100.00	OECD 203

Table 19. Acute aquatic toxicity of 3~32 PSU of the discharge treated water from BioViolet™

	Species	NOEC	LOEC	LC ₅₀ and/or EC ₅₀		Reference
		Conc. (%)		End point	Conc. (%)	
Invertebrate	<i>Brachionus plicatilis</i>	100.00	> 100.00	24h-LC ₅₀	> 100.00	ASTM E1440-91
Fish	<i>Paralichthys olivaceus</i>	100.00	> 100.00	96h-LC ₅₀	> 100.00	OECD 203

4.3.3 Chronic aquatic toxicity (Table 20, 21)

Invertebrate: Study to assess the effects of the discharge treated water from the BioViolet™ on the Rotifer, *B. plicatilis*, for 96 hours, NOEC, LOEC and 96h-EC₅₀ value based on population growth rate data at the end of the experiment were calculated for > 32 and 3~32 PSU discharge treated water from BioViolet™. > 32 and 3~32 PSU discharge treated water did not have chronic toxic effect on the Rotifer, *B. plicatilis*.

Fish: Study to assess the effects of the discharge treated water from BioViolet™ on the olive flounder, *P. olivaceus*, for 7 days, NOEC, LOEC, 7d-LC₂₅ and 7d-LC₅₀ value based on survival data at the end of the experiment were calculated for both > 32 and 3~32 PSU discharge treated water from the BioViolet™. > 32 PSU discharge treated water, NOEC, LOEC, 7d-LC₂₅ and 7d-LC₅₀ value of flounder, *P. olivaceus* chronic toxicity test were 50.00 %, 100.00 %, 80.50% and >100.00%, respectively. For 3~32 PSU discharge treated water, NOEC, LOEC, 7d-LC₂₅ and 7d-LC₅₀ value were equal to higher than 100.00 %.

Table 20. Chronic aquatic toxicity of > 32 PSU the discharge treated water from BioViolet™

	Species	NOEC	LOEC	LC _{25, 50} and/or EC ₅₀		Reference
		Conc. (%)		End point	Conc. (%)	
Invertebrate	<i>Brachionus plicatilis</i>	100.00	> 100.00	96h-EC ₅₀	> 100.00	- ASTM E1440-91 - Janssen <i>et al.</i> , 1994
Fish	<i>Paralichthys olivaceus</i>	50.00	100.00	7d-LC ₂₅ 7d-LC ₅₀	80.50 > 100.00	OECD 212

Table 21. Chronic aquatic toxicity of 3~32 PSU the discharge treated water from BioViolet™

	Species	NOEC	LOEC	LC _{25, 50} and/or EC ₅₀		Reference
		Conc. (%)		End point	Conc. (%)	
Invertebrate	<i>Brachionus plicatilis</i>	100.00	> 100.00	96h-EC ₅₀	> 100.00	- ASTM E1440-91 - Janssen <i>et al.</i> , 1994
Fish	<i>Paralichthys olivaceus</i>	100.00	> 100.00	7d-LC ₂₅ 7d-LC ₅₀	> 100.00 > 100.00	OECD 212

4.3.4 Endocrine disruption, sediment toxicity, bioavailability, biomagnifications, bioconcentration, food web, population.

4.3.4.1 Active substances

The BioViolet™ disinfects organism with physical and mechanical ways without using any active substance like chemical substance. Photon can't have any influence on aquatic environment because "Photon" exists only in the UV chamber not in the treated water. Therefore, this is not applicable to evaluation.

4.3.4.2 Relevant chemicals

DOM (dissolved organic matter), nitrate (NO₃-) and nitrite (NO₂-) of seawater, are reacted with ultraviolet ray, produces hydroxyl radical (·OH). ·OH is a strong oxidizing agent that has short lifetime about nanosecond. Therefore, it exists only in the UV chamber and it can't have any influence on aquatic environment. Therefore, this is not applicable to evaluation.

4.4 Data on mammalian toxicity (G9: 4.2.1.2)

4.4.1 Acute toxicity, effects on skin and eye, repeated-does toxicity, chronic toxicity, developmental and reproductive toxicity, carcinogenicity, mutagenic/genotoxicity

4.4.1.1 Active Substances

Photon which is an active substance of the BioViolet™ exists in the UV chamber during ballasting and de-ballasting processes. It can't have any influence on mammalian which live in aquatic environment. Therefore, this is not applicable to evaluation.

4.4.1.2 Relevant chemicals

Hydroxyl radical (·OH) is a strong oxidizing agent, that has short lifetime. So, it exists only in the UV chamber and it is not direct influence on mammalian. Therefore, this is not applicable to evaluation.

4.5 Data on environmental fate and effects under aerobic and anaerobic conditions (G9: 4.2.1.3)

4.5.1 Bioaccumulation, partition coefficient, octanol/water partition coefficient

4.5.1.1 Active substances

Photon which is an active substance of the BioViolet™ disinfects aquatic organisms by harm to DNA and RNA of cells. Photon exists only in the UV chamber during operation. Therefore, this is not applicable to evaluation.

4.5.1.2 Relevant chemicals

Hydroxyl radical ($\cdot\text{OH}$) is produced by photochemical reaction which dosed with the UV chamber. However, since hydroxyl radical has short lifetime about nanosecond, it exists only in the UV chamber during each operating process. Therefore, It doesn't exist in the ballast tank and treated water.

4.5.2 Persistence and identification of the main metabolites in the relevant media (ballast water, marine and fresh water)

Active substance (photon) and relevant chemical (hydroxyl radical, $\cdot\text{OH}$) of the BioViolet™ exists only in the UV chamber during operation. In addition, the other components during UV disinfection can be formed by photolysis and reaction with hydroxyl radical ($\cdot\text{OH}$). Natural water which includes predominantly NOM and nitrate ion absorbs mainly at the lower wavelengths in the UV-C region (200~280 nm). Photolysis of natural water results in the breaking of bonds within organic molecules creating smaller organic molecules. The other components were measured by the Chemical Analysis (appendix 5)

4.6 Physical and chemical properties for the active substances and preparations and the treated ballast water (G9:4.2.1.4)

4.6.1 Active substance

Information about these was not found anywhere.

4.6.2 Relevant chemicals

Table 22 shows Physical and chemical properties of hydroxyl radical ($\cdot\text{OH}$).

Table 22. Physical and chemical properties of hydroxyl radical($\cdot\text{OH}$)

Property	Value	Reference
Melting point (°C)	146.26 °C (MPBPVP v. 1.43)	EPI Suite, 2012
Boiling point (°C)	439.86 °C (MPBPVP v. 1.43)	EPI Suite, 2012
Flammability (flash point for liquids; °C)	-	-
Density (20°C; kg/m ³)	-	-
Vapour pressure (20°C; Pa)	1.36 E-007 mmHg at 25 °C (MPBPVP v. 1.43)	EPI Suite, 2012
Vapour density (air = 1)	-	-
Water solubility (temp; effect of pH; mg/L)	1 E+006 mg/L at 25 °C (WSKOW v. 1.41)	EPI Suite, 2012
pH in solution	-	-
Dissociation constant (pKa)	-	-
Oxidation-reduction potential	2.80 V	IMO, 2011(63/2)
Corrosivity to material or equipment	-	-
Reactivity to container material	-	-
Auto-ignition temperature (°C)	-	-
Explosive properties	-	-
Oxidizing properties	Non-selective, various routes	IMO, 2010 (62/2/9)
Surface tension (newtons/m (SI) or dynes/cm (CGS))	-	-
Viscosity (mPa-s)	-	-
Henry's law constant (Pa.m ³ /mol)	8.52 E-004 Pa-m ³ /mol (HENRYWIN v. 3.20)	EPI Suite, 2012
Partition coefficient (Koc)	0.06337 (KOCWIN v. 2.00)	EPI Suite, 2012
Log Pow (Kow)	- 1.38 (KOWWIN v. 1.68)	EPI Suite, 2012

5. USE OF THE ACTIVE SUBSTANCE OR THE PREPARATION

5.1 System components and operations

BioViolet™ is composed of filtration system, UV system, and control system. Aquatic organisms are removed via filtration system and UV system. The control system of the test facility uses flow information acquired from flow meter during ballasting and de-ballasting processes for real-time control of ballast pump to maintain flow at $250 \pm 5 \text{ m}^3/\text{h}$ during each operation process.

Filtration system strains out most of aquatic organisms and particles in ballast water coming in through ballast pump larger than $50\mu\text{m}$, both increasing disinfection efficiency of BWMS and preventing breakage of quartz tube that protects UV lamp. In such filtration process, sediment that influences UV transmittance is removed and physical impact is inflicted on aquatic organisms passing through the filter, increasing overall disinfection efficiency of BioViolet™.

Filtration system is installed between ballast pump and UV system. It is only used in ballasting operation and by-passed in de-ballasting operation.

The UV system disinfects aquatic organisms that had not been removed by the filtration process during ballasting operation and is used once again during de-ballasting operation to disinfect aquatic organisms that can potentially survive in the ballast tank.

For testing of brackish water (3~32 PSU) and seawater ($> 32 \text{ PSU}$), the UV system was operated twice for each test at maximum power to conduct chemical analysis, aquatic toxicity test and efficacy test under maximum UV dose. In addition for remaining tests, power of the UV lamp was controlled to maintain constant UV intensity value for examination of disinfection efficiency.

The control system controls and monitors the entire BioViolet™. It stores all records during operation. Also, it exchanges important data including alarms with the central control system through communication. The control system shuts down the system if severe level of abnormality is detected.

Refer to “Appendix 1” for detailed system description.

5.2 Use of the active substance or the preparation

BioViolet™ only uses active substances in the UV chamber. The UV system disinfects aquatic organisms by the “photon” irradiated from the UV lamp by damaging DNA and RNA. Also, DOM, nitrate (NO_3^-), and nitrite (NO_2^-) in water form $\cdot\text{OH}$ by photochemical reaction with UV light irradiated from the UV lamp. $\cdot\text{OH}$ is a strong oxidant with extremely short lifetime of nanoseconds, but it occurs in extremely small amounts.

Since active substance and relevant chemical have short lifetime of nanoseconds, they do not exist in treated water that passed through the UV system. Therefore, these substances have no influence on the environment and human body.

The UV system of BioViolet™ is designed with a sealed structure in which photons cannot be exposed outside the chamber during operation process unless the chamber is disassembled. Hence, there is no risk of photon exposure.

Refer to “8. Risk Assessment” for details on safety.

6. MATERIAL SAFETY DATA SHEETS (G9: 4.2.7)

Filter of the BioViolet™ removes aquatic organisms (> 50 um) and particles. If aquatic organisms are un-removed with filter, are disinfected by the UV irradiation. These processes do not include chemical elements as a chemical-free disinfection system. Therefore, this is not applicable to evaluation.

7. RISK CHARACTERIZATIONS

7.1 Screening for persistency, bioaccumulation and toxicity (PBT) (G9:5.1)

The BioViolet™ disinfects aquatic organisms with physical and mechanical ways without using any active substance like chemical substance. Photon exists only in the UV chamber not in the treated water. Also, DOM (dissolved organic matter), nitrate (NO_3^-) and nitrite (NO_2^-) of seawater, are reacted with ultraviolet ray, produces hydroxyl radical. $\cdot\text{OH}$ is a strong oxidizing agent that has short lifetime about nanosecond. Therefore, it exists only in the UV chamber and it can't have any influence on aquatic environment. Therefore, this is not applicable to evaluation

7.2 Toxicity testing

See appendix 5 of the confidential dossier.

7.3 Risk characterization and analysis

7.3.1 Reaction with organic matter

UV irradiation is known to be more effective than UV-C irradiation in formation of biodegradable compounds and mineralization (Buchanan et al., 2004). UV-A and UV-B can also split large NOM molecules into organic acids with lower molecular weight (Frimmel, 1998). This change of DOM structure can increase biodegradability, which stimulates microbial re-growth and biofilm formation in distribution system. Also UV treatment is often expected to reduce DBPs formation. And the effect of UV irradiation on water quality depends on many factors, such as characteristics of source water quality, UV wavelength and applied dosage. (Yonkyu Choi et al., 2010)

- Source : The effects of UV disinfection on drinking water quality in distribution systems (Choi et al., 2010)

7.3.2 Prediction of discharge and environmental concentrations, assessment of potential for bioaccumulation, effects assessments, effects on aquatic organisms, effect on sediment

The BioViolet™ disinfects aquatic organisms with physical and mechanical ways without using any active substance like chemical substance. So, These processes does not include chemical elements as a chemical-free disinfection system.

Therefore, this is not applicable to evaluation.

8. RISK ASSESSMENT

8.1 Risk to safety ship

8.1.1 Corrosion

The main factors influencing the progress of corrosion of mild steel in seawater are; salinity, pH, water temperature, dissolved oxygen content, water velocity, sulphide pollution and bio-fouling. Corrosion in ship structures also proceeds in the presence of both open and enclosed atmospheric environment (Gardiner and Melchers, 1999). A state-of-the-art review of atmospheric corrosion research is given by Strekalov (Strekalov, 1998). The main influencing variables are; salt deposition, time of wetness (relative humidity) and temperature.

- Source: Corrosion analysis of bulk carriers, Part I: operational parameters influencing corrosion rates (Gardiner, 2003).

In comparison of test water and treated water, if changes in the above factors are observed, they may be considered to have influence on corrosion in BioViolet™. However, values such as pH, salinity, and DO verified through efficacy test were similar. And if TRO (total residual oxidant) is formed by treatment process of BioViolet™, ORP (oxidation-reduction potential) of the treated water must increase by oxidation reaction of such TRO. However, no change in ORP value was observed.

Thus, there is no additional risk of ship's corrosion by the use of BioViolet™.

8.1.2 Fire and explosion

BioViolet™ does not use additional chemical compounds for disinfection throughout the entire system. Since it disinfects organisms using photons radiated by the UV lamp as active substances, there is no risk of fire and explosion at the reactor.

However, electric system composed of ballast, power distributor, and control system have potential risk of fire and explosion. Overall electric system of BioViolet™ is equipped with safety devices (overload protection, short circuit protection, etc.) in order to overcome such risks.

The BioViolet™ controller always monitors ballast and power system. When overload or short circuit is observed in them, the controller immediately shuts down the system and notifies relevant information to the operator by using annunciator and logging data.

8.1.3 Storage and handling of the substance

Since BioViolet™ does not use additional chemical compounds for disinfection throughout the entire system, storage and handling of the substance need not be taken into consideration.

8.1.4 Contact with, or Inhalation of, Process Product

Since there is no active substance and relevant chemical in the treated water that passed through the UV system during ballasting and de-ballasting operation, there is no influence on human body. Also since the UV system is designed with a sealed structure in which photons cannot be exposed outside the chamber unless the chamber is disassembled, photon will not come into contact with the crew.

8.1.5 Noise

During operation, BioViolet™ does not create electric noise at the level that influences other systems or physical sound created by the fluid passing through the system.

8. 2 Risk to human health

8.2.1 Health effects in humans

Filter of the BioViolet™ removes aquatic organisms (> 50 um) and particles. If aquatic organisms are un-removed with filter, are disinfected by the UV irradiation. Photon which is an active substance of the BioViolet™, exists in the UV chamber during operating processes. However, since hydroxyl radical has short lifetime about nanosecond, it exists only in the UV chamber during each operating process. Therefore It doesn't exist in the ballast tank and treated water.

Therefore, the BioViolet™ can't have influence to human health of the general public, crew and passengers.

8.2.2 Human Exposure Scenario (HES)

As mentioned earlier, the BioViolet™ can't have influence to human health of the general public, crew and passengers. Therefore, HES is not applied to the BioViolet™.

8.3 Risk to aquatic environment

Photon exists only in the UV chamber during operating process and hydroxyl radical has short lifetime about nanosecond. Therefore, it exists only in the UV chamber and it can't have any influence on aquatic environment.

9. REFERENCES

- Bolton, J.R., and Cristine, C.A. (2008). The ultraviolet disinfection handbook. American Water Works Association Research Foundation, Denver, Colo.
- Choi, Y.K., Choi, Y.J. (2010). The effects of UV disinfection on drinking water quality in distribution system. *Water Research*. 44(1), 115-122.
- Cooper, W.J., Zika, R.G., Petsane, R.G., Plane, J.M.C. (1988). Photochemical formation of H₂O₂ in natural waters exposed to sunlight. *Environmental Science and Technology*. 22(10), 1156-1160.
- Edhlund, B.L. (2008). Natural water photochemistry: Singlet oxygen production and the degradation of dissolved organic nitrogen and organic pollutants. University of Minnesota, *Ph. D. Thesis*, 146 p.
- Gardiner, C.P., Melchers. R.E. (2003). Corrosion analysis of bulk carriers, Part I: Operational parameters influencing corrosion rates. *Marine Structures*. 16(8), 547-566.
- Griffiths, H.R., Mistry, P., Herbert, K.E., Lunec, J. (1998). Molecular and cellular effects of ultraviolet light induced genotoxicity. *Critical Reviews in Clinical Laboratory Sciences*. 35(3), 189-237.
- Holzinger, A., Lutz, C. (2006). Algae and UV irradiation: Effects on ultrastructure and related metabolic functions. *Micron*. 37(3), 190-207.
- Kieber, D. J., Peake, B.M., and Scully, N.M. (2003). Reactive oxygen species in aquatic ecosystems. In *UV effects in aquatic organisms and ecosystems*, ed. E.W. Hebling and H. Zagarese, pp. 251-288. Royal Society of Chemistry, Cambridge.
- Knudson, G.B. (1985). Photoreactivation of UV-irradiated *Legionella pneumophila* and other *Legionella* species. *Applied and Environmental Microbiology*. 49(4), 975-980.
- Kowalski, W.J. (2009). Ultraviolet germicidal irradiation handbook: UVGI for air and surface disinfection. Springer, New York.
- Kruithof, J.C., and van der Leer, R.C. (1990). Practical experiences with UV-disinfection in the Netherlands. Proceedings of the American Water Works Association seminar on emerging technologies in practice, Annual Conference of the American Water Works Association, June 17-21, Cincinnati, OH.
- Larson, R.A., and Berenbaum, M.R. (1988). Environmental phototoxicity: Solar ultraviolet radiation affects the toxicity of natural and man-made chemicals. *Environmental Science and Technology*. 22(4), 354-360.
- Li, J., Uchida, T., and Kitagawa, T. (2006). Similarities and differences between cyclobutane pyrimidine dimer (CPD) photolyase and (6-4) photolyase as revealed by resonance raman

spectroscopy: Electron transfer from FAD cofactor to UV-damaged DNA. *Journal of Biological Chemistry*. 281(35), 25551-25559.

Macfadyen, E.J., Williamson, C.E., Grad, G., Lowery, M., Jeffrey, W.H., and Mitchell, D.L. (2004). Molecular response to climate change: Temperature dependence of UV-induced DNA damage and repair in the freshwater crustacean *Daphnia pulicaria*. *Global Change Biology*. 10(4), 408-416.

MacKinnon, M.D. (1981). The measurement of organic carbon in sea water. In *Marine organic chemistry*, ed. Duursma, E.K., Dawson, R., pp. 415-443. Elsevier Scientific Publishing, Amsterdam.

Mitchell, D. L., and Karentz, D. (1993). The induction and repair of DNA photodamage in the environment. In *Environmental UV photobiology*, ed. Young, A.R., Björn, L.O., Moan, J., and Nultsch, W. Plenum Press, New York.

Mitchell, D.L., Narin, R.S. (1989). The biology of the (6-4) photoproduct. *Photochemistry and Photobiology*. 49(6), 805-819.

Mopper, K., and Zhou, X. 1990. Hydroxyl radical photoproduction in the sea and its potential impact on marine processes. *Science New Series*. 250, 661-664.

Rauth, A.M. (1965). The physical state of viral nucleic acid and the sensitivity of viruses to ultraviolet light. *Biophysical Journal*. 5(3), 257- 273.

USEPA. (1999). Alternative disinfectants and oxidants guidance manual. EPA 815-R-99-014. U.S. Environmental Protection Agency, Office of Water, Washinton, DC.
<http://www.epa.gov/safewater/mdbp/mdbptg.html#disinfect>

USEPA. (2006). Ultraviolet disinfection guidance manual for the final long term 2 enhanced surface water treatment rule. EPA 815-R-06-007. U.S. Environmental Protection Agency, Office of Water, Washinton, DC.
<http://www.epa.gov/safewater/disinfection/lt2/compliance.html>

Yoon, C.G., Jung, K.W., Jang, J.H., Kim, H.C. (2007). Microorganism repair after UV-disinfection of secondary-level effluent for agricultural irrigation. *Paddy and Water Environment*. 5(1), 57-62.

Zimmer, J.L., Slawson, R.M. (2002). Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. *Applied and Environmental Microbiology*. 68(7), 3293-3299

Zheng, M., Andrews, S.A., Bolton, J.R. (1999). Impacts of medium pressure UV on THM and HAA formation in pre-UV chlorinated drinking water. Proceedings of the American Water Works Association Water Quality Technology Conference, October 31-November 3, Tampa, FL.

Zika, R.G., Moffett, J. Petasne, R., Cooper, W.J., Saltzman, E. (1985). Spatial and temporal variations of hydrogen peroxide in Gulf of Mexico waters. *Geochimica et Cosmochimica Acta*. 49(5), 1173-1184.

Zimmer, J.L., Slawson, R.M. (2002). Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. *Applied and Environmental Microbiology*. 68(7), 3293-3299.